



ProFoldin

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INSTRUCTIONS

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Human DNA Topoisomerase I Assay Kits

Human DNA Topoisomerase I Assay Kit

Catalog No. HRA020K

Human DNA Topoisomerase I Assay Kit-100

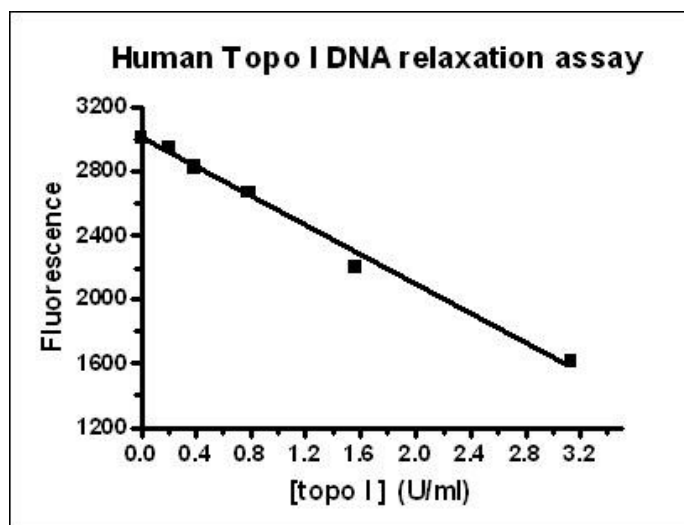
Catalog No. HRA100K

H19 Dye for DNA Relaxation and Supercoiling Assays

Catalog No. DSA1000D

Introduction

Human DNA topoisomerase I converts supercoiled circular DNA into relaxed DNA (DNA relaxation reaction). The **Human DNA Topoisomerase I Assay Kit** is based on the principle that the relaxed DNA suppresses the fluorescent intensity much more than the supercoiled DNA when the DNA interacts with fluorescence dye H19 (a fluorescence dye for DNA relaxation / supercoiling assay) in the presence of magnesium. When the supercoiled DNA is converted into its relaxed form, the fluorescent signal decreases.



The **Human DNA Topoisomerase I Assay Kit** (Catalog No. HRA020K) includes 160 μ l of Buffer HT, 84 μ l of 10 x supercoiled plasmid DNA, 8 μ l of 1500 x Dye H19 and 550 μ l of 10 x H19 dilution buffer. It is for 20 assays of DNA relaxation reactions in a 96-well plate format or 40 assays in a 384-well assay format. The kit does not include human topoisomerase I.

The **Human DNA Topoisomerase I Assay Kit-100** (Catalog No. HRA100K) includes 480 μ l of Buffer HT, 405 μ l of 10 x supercoiled plasmid DNA, 20 μ l of 1500 x Dye H19 and 3000 μ l of 10 x H19 dilution buffer. It is for 100 assays of DNA relaxation reactions in a 96-well plate format or 200 assays in a 384-well assay format. The kit does not include human topoisomerase I.



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The **H19 Dye for DNA Relaxation and Supercoiling Assays** (Catalog No. DSA1000D) includes 170 μ l of 1500 x Dye H19 and 26 ml of 10 x H19 dilution buffer. It is for 1000 assays of DNA relaxation or supercoiling reactions in a 96-well plate format.

Assay Protocol

1. Reaction and sample preparation:

The total volume of each reaction mixture is 40 μ l including: 28 μ l of H₂O, 4 μ l of 10 x Buffer HT, 4 μ l of 10 x supercoiled DNA, 4 μ l of 10 x Topoisomerase I. Incubate the reaction mixture at room temperature for 60 min.

Note: The final concentrations are 10 mM Tris-HCl, pH 8, 50 mM NaCl, 0.1 mM EDTA, 50 mM KCl, 5 mM MgCl₂, 15 μ g/ml BSA, 25 μ g/ml supercoiled plasmid DNA and human topoisomerase I.

Human topoisomerase I is commercially available from Sigma (catalog number: T9069) or other vendors. The human topoisomerase concentration in the assay is optimized by testing different concentrations of the enzyme in the assay. Typically 10 to 20 U/ml of human topoisomerase I is used. One unit is the enzyme quantity for relaxation of 0.25 μ g of supercoiled DNA in 30 min at 37°C.

Free magnesium is required for the H19 detection of the DNA topology. High concentrations of EDTA should be avoided in the reaction and detection solution.

2. Assay

- (1) Make 1x H19 Dilution Buffer by dilution of the 10 x H19 Dilution Buffer 10-fold with water. Freshly prepare the H19 dye by dilution of 1 μ l the 1500x H19 dye stock solution with 1.5 ml of 1x H19 Dilution Buffer (1500 x dilution).
- (2) Mix 250 μ l of the diluted H19 dye with each reaction solution. Incubate the mixture for 5 min.
- (3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Related Products:

Human Topoisomerase II DNA Decatenation Assay Kit Plus-100	Catalog No. HDC100KE
96-Well Human Topo II DNA Decatenation Assay Kit Plus	Catalog No. HDD96KE
E. coli DNA Topoisomerase I Assay Kit Plus-100	Catalog No. DRA100KE
E. coli DNA Topoisomerase IV Assay Kit Plus-100	Catalog No. DDC100KE
Supercoiled Plasmid DNA -1 mg	Catalog No. SDNA-1MG
Supercoiled Plasmid DNA -10mg	Catalog No. SDNA-10MG

For more information of DNA topoisomerase assays, please see the website of The Topo World: http://www.profoldin.com/topoisomerase_assays_1.html. For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.